Chapter 3

Hunger regulates the dispersal of *Drosophila* from food

3.1 Summary

Assessing the quality of a feeding site and deciding whether to move to a site of possibly higher quality food is an important and constant decision in an animal's life. Cues from both an animal's physiology and the environment influence the decision to disperse from identified sites containing food. Hunger has been implicated as a important factor influencing the search behavior of most motile animals (Barton Browne, 1993) and has been frequently studied in insects (Bell, 1990).

We have used a system of environmental chambers to carry out laboratory experiments from which we suggest that hunger regulates the dispersal of the fruit fly, *Drosophila melanogaster*, independent of sensory cues arising from food. As expected, food inhibited the dispersal of hungry flies; however, hungry flies dispersed from detectible, yet inaccessible food at a similar elevated rate as they dispersed from a chamber containing only water. Further, sated flies dispersed at a low and similar rate irrespective of the presence or accessibility of food. Flies homozygous for different alleles of the *forag-*

ing gene, noted for differences in their locomotor behavior on and around food, were comparable in their dispersal from food. Results from experiments measuring a fly's general locomotor activity indicate that a change in the intensity of activity was insufficient for explaining the hunger-induced dispersal. From these experiments we suggest that the hunger state of flies can override the visual and olfactory cues from food; we hypothesize that the observed increase in dispersal resulting from hunger was due to a qualitative change in locomotor behavior related to food search.

3.2 Introduction

Hungry flies, like various other insects deprived of food, behave in ways to increase their probability of finding and consuming food (Bell, 1985; Barton Browne, 1993). Detailed studies indicate that the movement before and after feeding is similar among blow flies, *Phormia sp.* (Dethier, 1957, 1976; Nelson, 1977), house flies, *Musca sp.* (Mourier, 1964; White et al., 1984), and fruit flies, *Drosophila* (Bell et al., 1985; Mayor et al., 1987). *Phormia*, *Musca*, and *Drosophila* have been described to move in relatively straight paths at moderate speeds until they come upon a patch of food (Dethier, 1957; Mourier, 1964; Bell et al., 1985). Upon finding food, it has been reported that their locomotor rate decreases and their turning rate increases (Bell et al., 1985), that eventually they stop, and if the food is acceptable, they feed (Nelson, 1977; White et al., 1984). Upon finding a sufficiently large patch of food, several studies report that flies stop and eat until satiety, afterwards moving very little (Green, 1964a; Dethier, 1976; Bell et al., 1985). After feeding, as flies become hungry, their movement has been characterized as speeding up and straightening out (Dethier, 1957; White et al., 1984; Bell et al., 1985), thereby displacing them from the site of food (White et al., 1984). Many

studies have shown that hungry flies search longer, farther, and more intensely on and around patches of food (Dethier, 1957; Mourier, 1964; Nelson, 1977; Bell et al., 1985; Mayor et al., 1987). In particular it has been observed that as *Drosophila* become hungrier, they return to food in greater numbers and also stay closer to food (Mayor et al., 1987). We have no prior knowledge of how flies respond to detectable, yet inaccessible food.

The primary goal of this work was to investigate to what extent hunger influenced the dispersal of flies from a patch of food, independently from sensory cues from food. Additionally, we considered whether a change in the intensity of a fly's general locomotor activity was sufficient to explain the observed effects of hunger. We carried out these studies within the laboratory using a custom-built system of connected chambers. This technology allowed us to systematically manipulate features of simplified environments while automatically quantifying the movement of *Drosophila*.

3.3 Results

3.3.1 Hunger regulates dispersal

To examine and characterize to what extent hunger influences the dispersal of flies from patches of food, we carried out a series of experiments using environmental test chambers that we have described previously. As expected, hungry flies introduced to a chamber containing food dispersed to a second chamber at an inhibited rate compared to when they left only water (Fig. 3.1). Their emigration rate was significantly different whether dispersing from water, 65 μL food, or 100 μL food (Kruskal-Wallis, p = 0.0001), with the 100 μL patch inhibiting dispersal more than the 65 μL patch (0.7 $\pm$ 0.7 exit h^{-6} vs.

2.4 ± 1.1 exit h^{-6}, T-test, $p < 0.0001$). However, sated flies that were given food *ad libitum* for 12 hours prior to the start of the experiment dispersed at a low and comparable rate whether or not food was present in the first chamber (Fig. 3.1). This dispersal was significantly lower than that of hungry flies from water (Mann-Whitney U, $p < 0.0001$). Taken together, these results suggest that dispersal is triggered by hunger, and not by the limited availability of food.

To test more directly whether odor or visual cues associated with food might inhibit dispersal, we placed food in chambers beneath a mesh so that the flies could see and smell the food but could not touch or consume it. Hungry flies dispersed from the inaccessible food at a similar elevated rate (2.2 ± 0.4 exit h^{-6}) as they dispersed from a source of water (accessible, 2.6 ± 0.7 exit h^{-6}, covered, 2.1 ± 0.3 exit h^{-6}) (Fig. 3.2A, B, D-F, and H; ANOVA, $p = 0.691$). This rate was significantly greater than that of flies dispersing from accessible food (Fig. 3.2; ANOVA, $p = 0.002$; Tukeys HSD, significance level of 0.05). As observed before, sated flies dispersed at a rate (Fig. 3.2A-H; ANOVA, $p = 0.280$) that was significantly lower than hungry flies, irrespective of the presence or accessibility of food (Fig. 3.2A-H; Collective dispersal for hungry and sated flies from all resources, Mann-Whitney U, $p < 0.0001$). These results suggest that hunger drives dispersal despite the attractive sensory stimuli associated with food.

The elevated dispersal observed for flies in the presence of inaccessible food might be explained by the flies' inability to detect the food. To verify that flies could detect the presence of the inaccessible food, we introduced flies into a single chamber with food placed beneath a mesh cover and observed their behavior directly from recorded digital video. To help visualize the behavior of these flies, we modified our basic design in two ways. First, we blocked the exit from the chamber. This prevented the flies from dispersing and therefore allowed us to observe their behavior over long periods

of time. Second, we excluded accessible water. We found that the majority of the flies would congregate near water, diminishing our ability to determine whether or not they could detect the inaccessible food (data not shown). Our results show that a significantly greater number of flies loitered over water and food than they did over water alone (Fig. 3.3). We observed this difference whether we examined the full-length experiment or just the first two hours. (Full experiment, Mann-Whitney U, $p < 0.0001$; First two hours, Mann-Whitney U, $p = 0.04$, 1-tailed.) The number of flies loitering over water and food and water alone were both much greater than the number of flies loitering over empty cuvettes (Fig. 3.3G-J), as expected for flies deprived of both food and water. These results suggest that flies could detect the amount of food placed beneath the mesh that was used during the dispersal experiments, and that the food remained attractive to hungry flies if flies were confined to an area and not permitted to disperse.

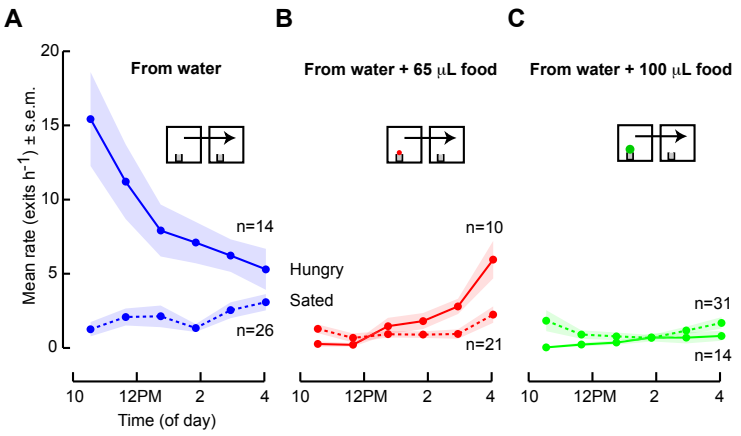

Figure 3.1: Hunger inhibits dispersal from food. Food in a first chamber inhibits the dispersal of hungry flies (solid) to a second chamber containing only water. (A) Mean $\pm$ s.e.m. exit h^{-1} dispersal rates from chambers containing only water (solid blue, 2 mL 0.5% agar). (B, C) The mean dispersal rate was significantly greater from chambers containing 65 μL of food on 2 mL 0.5% agar (solid red) than 100 μL of food on 2 mL 0.5% agar (solid green). (A-C) Sated flies (dashed) dispersed at a significantly lower rate than hungry flies (solid). This rate was comparable whether or not food was present in the first chamber.

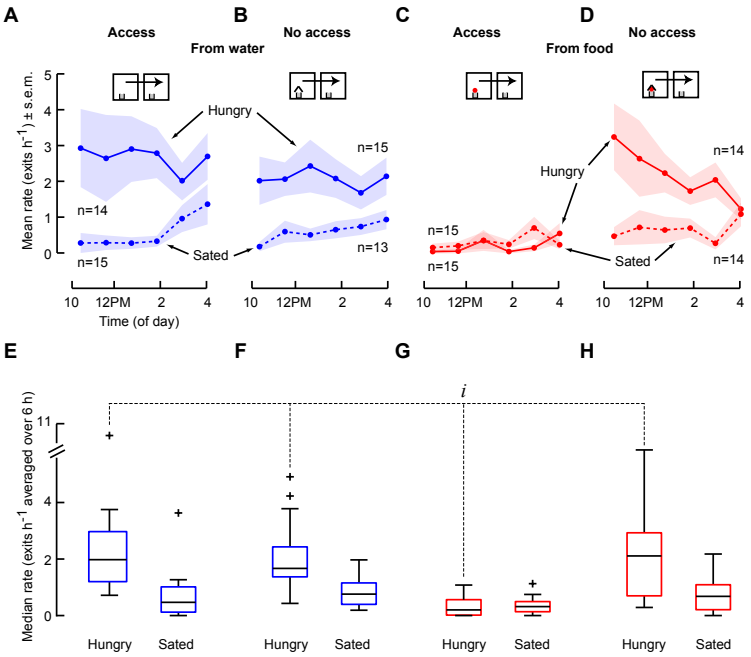

Figure 3.2: Hunger and not cues from food inhibits dispersal. Hungry flies dispersed at a similar rate from chambers in which food cues are present, but access to food was inhibited (D, solid red, H, 65 μL on 2 mL 0.5% agar beneath a mesh) and from chambers in which food was absent (A, solid blue, E, 2 mL 0.5% agar; B, solid blue, F, 2 mL 0.5% agar beneath a mesh). This rate was significantly greater than the dispersal of hungry flies from chambers in which food was accessible (C, solid red; G, indicated by *i*). (A-H) Sated flies (dashed) dispersed at a similar rate irrespective of the presence or accessibility of food in the first chamber, and this rate was significantly lower than hungry flies. (E-H) Median hourly rates averaged over 6 hours. The top and bottom edges of the boxes represent 75^{th} and 25^{th} percentiles; the whiskers extend to the most extreme point not considered outliers, and outliers are plotted individually (+).

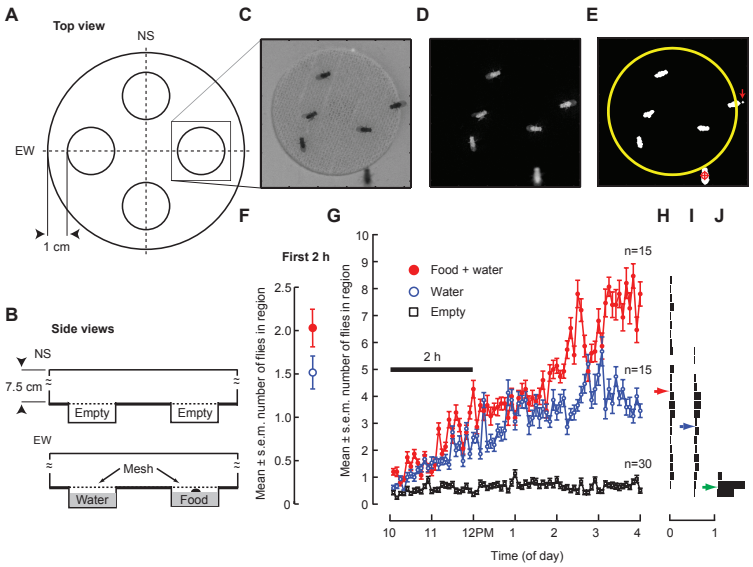

Figure 3.3: Hungry flies can detect the presence of food embedded in the floor covered beneath a mesh. (A) Schematic showing the possible locations of cuvettes containing food. (A, B) North-South (NS) and East-West (EW) cross-sections illustrate a possible configuration of the cuvettes containing either water and a patch of food, only water, or nothing. (C) Cropped region from a digital video sequence showing the typical number of flies observed loitering over a mesh covering a cuvette containing water and a patch of food. (D) Image from C minus a background image made from the average of 10 frames of video from the same region. (E) Thresholded image from D used to determine the location of flies within this region. We tallied the number of flies loitering within the specified region (yellow circle). To avoid false counts, we excluded objects that were larger and smaller than the range of pixel areas that could reasonably include flies. For example, a fly sitting upside down on the lid of the chamber had a pixel area too large and was not counted, as shown by the fly at the bottom of the image (red crosshairs). Small objects due to digital noise or processing errors, as shown by the fly's wings in the right of the image, were also not counted (red arrowhead). (F) Flies loitered over water and a patch of food (65 μL on 2 mL 0.5% agar, red closed circle) to a significantly greater extent than over only water (2 mL 0.5% agar, blue open circle) during the first 2 hours of the experiment. (G) Mean $\pm$ s.e.m. number of flies loitering over water and a patch of food (65 μL on 2 mL 0.5% agar, red closed circles), only water (2 mL 0.5% agar, blue open circles), and nothing (black squares) over the length of the experiment. Evidence that a greater number of flies loitered over water and food than water alone became stronger if we compared loitering during the full experiment. (H) Histograms showing the mean frequency of flies loitering over water and food, (I) only water, (J) and empty containers during the full experiment. We show the mean number of flies loitering over water and food (red arrowhead), only water (blue arrowhead), and empty containers (green arrowhead).

3.3.2 Activity does not explain dispersal

One possible explanation is that the increased rates of dispersal observed in hungry flies could be explained by a change in the level of their general activity. To test this hypothesis, we introduced individual hungry and sated flies into activity monitors and measured their activity for 6 hours. We repeatedly did not observe an increase in the level of their activity. Females showed a similar level of activity whether they were hungry or sated (hungry, 5.4 $\pm$ 0.7 beam crosses h^{-6}, n = 22; sated, 4.8 $\pm$ 0.5 beam crosses h^{-6}, n = 19; T-test, p = 0.503) and males were less active when deprived of food (hungry, 4.9 $\pm$ 0.7 beam crosses h^{-6}, n = 24; sated, 8.3 $\pm$ 1.0 beam crosses h^{-6}, n = 26; Mann-Whitney U, p = 0.010). Results from these experiments suggest that the greater level of dispersal observed for hungry flies has a more complicated explanation than a change in the level of general activity, as measured with the widely utilized *Drosophila* activity monitors. Rather, the activity arises from an increase in search behavior that is not detectable in the simple geometry of an activity monitor.

3.4 Discussion and conclusions

Using a system of connected environmental chambers, we have shown that hunger alone, and not cues emanating from food, regulates the dispersal of *Drosophila*. Hungry flies rapidly left chambers containing only water, whereas the presence of accessible food inhibited their dispersal (Fig. 3.1, 3.2). The rate of dispersal varied according to the amount of food present; the greater the amount of food within a chamber, the slower the flies dispersed from it (Fig. 3.1). A key observation in this study was that hungry flies dispersed from detectible, though *inaccessible* food at a similar elevated rate as if they were dispersing from only water (Fig. 3.2). This implies that the sensory stimuli

originating from food do not inhibit dispersal. The importance of hunger, and not stimuli related to food in controlling dispersal, is further supported by the behavior of sated flies, which dispersed between connected chambers at a low and similar rate irrespective of the presence, amount, or accessibility of food (Fig. 3.1, 3.2). Collectively, these experiments suggest that to initiate dispersal the hunger state of flies can override the visual and olfactory cues from food.

To the best of our knowledge, a hungry fly's response to detectible but inaccessible food is unknown. Previous studies report that flies presented with and consuming only a small amount of food searched afterward in loops and spirals (Dethier, 1957) within a 5≈6 cm diameter region (Nelson, 1977; Bell et al., 1985) around the area that contained the food patch (Mourier, 1964; Mayor et al., 1987). This convoluted movement has been reported to be remarkably similar in *Phormia*, *Musca*, and *Drosophila* (Murdie and Hassell, 1973; White et al., 1984). It is unlikely, however, that such a response could explain the elevated dispersal we have observed of hungry flies from inaccessible food (Fig. 3.2). It has been reported that hungry flies foraging without finding food stop less often (Dethier, 1957) and forage for relatively greater amounts of time than sated flies (White et al., 1984). It is therefore possible that a change in the level of a fly's general locomotor activity might explain its regulated dispersal. However, blood-borne factors associated with hunger and saity that have been shown to regulate the general locomotor activity of blow flies (Green, 1964b) did not affect their food searching response. Unfed parabiotic blow flies, pairs of flies that have been surgically connected so they share haemolymph, continued the searching response after their partners had fed and stopped searching, suggesting that this behavior is not simply a by-product arising from hormonally controlled changes in general locomotor activity (Nelson, 1977).

An important issue for this study is whether the regulated dispersal due to hunger

results from a general increase in locomotor activity or, alternatively, is due to a transition to a specific locomotor mode related to food search. Although our experiments cannot test these alternatives, our results do suggest that a change in general locomotor activity alone was insufficient to explain dispersal. In our studies, during the same 6 hour time window as we had run the dispersal experiments, we observed a steady and similar rate of locomotor activity for sated and hungry females and a decreasing rate of locomotor activity in hungry males, presumably as they began to dehydrate (see Results section on activity). Previous studies have reported that locomotor activity increases with food deprivation, but these observations are difficult to directly compare with our results (Connolly, 1966a; Bell et al., 1985). In several of these studies, the authors sampled short, less than 5 minute periods of movement. The recent handling of the flies possibly affected the results of such experiments. Knoppien and colleagues (Knoppien et al., 2000) measured the locomotor activity of food-deprived flies over a longer period of time and reported a steady level of higher activity instead of a graded, increasing level of locomotor activity. Martin (Martin, 2004) continually measured the locomotor activity of flies and found that as sated flies become hungry, they spend more time moving and move greater distances, but their activity reaches a maximum steady level after 2 hours. During our studies, we allowed flies 1 hour to settle down in the chambers before recording their activity. We assume that this time, plus the additional time taken to introduce each fly individually into the activity monitors (0$\approx$45 min), explains why we observed a steady, elevated level of activity in sated flies as opposed to an increasing level of activity as has been reported previously (Martin, 2004). From these results, we hypothesize that a change in a fly's general level of locomotor activity, as assayed in the *Drosophila* activity monitors, cannot directly explain the increased rates of dispersal that we have observed in hungry flies. One possible explanation is that the behavior

recorded in the small activity monitor represents an escape response to the confined space, which supersedes the locomotor response due to hunger.

3.4.1 Genetic contribution

Flies that posses *rover* or *sitter* (Osborne et al., 1997), allelic forms of the *foraging* gene that have been shown to exhibit significant differences in the flies' movement on and around food (Pereira and Sokolowski, 1993), dispersed at comparable rates from food. We report that groups of *rover* flies exhibited a sometimes similar, but overall lower rate of dispersal from food (1.5 $\pm$ 0.4 exit h^{-6}, n = 15) than sitters (3.1 $\pm$ 0.4 exit h^{-6}, n = 16)(T-test, p = 0.003). In contrast, however, we observed that groups of *rover* flies moved at a greater rate (forward, 7.9 $\pm$ 0.9 exit h^{-6}, return, 4.9 $\pm$ 0.8 exit h^{-6}, n = 20) between chambers containing only water than *sitters* (forward, 4.9 $\pm$ 0.5 exit h^{-6}, return, 2.9 $\pm$ 0.4 exit h^{-6}, n = 20)(Forward, T-test, p = 0.005; reverse, T-test, p = 0.038). This finding was consistent with a non-significant trend observed over a shorter time period reported previously (Pereira and Sokolowski, 1993).

3.4.2 Concluding remarks

We have designed and built a flexible system of hardware and software able to regulate and monitor the movement of groups of flies between controlled sensory environments in the laboratory. Through a series of experiments, we provide evidence suggesting that hunger regulates the dispersal of *Drosophila* independently of stimuli arising from food. Furthermore, a change in the level of the flies' general locomotor activity cannot directly explain hunger-induced dispersal. We require a richer description of dispersal before making conclusions regarding the mechanisms underlying the various factors

contributing to this complex behavior. It would be both informative to directly observe the movement of individual sated flies as they become hungry and to monitor in greater detail the response of flies deprived of food as they disperse from patches of accessible and inaccessible food. This is a direction of research that we are currently pursuing.

3.5 Materials and Methods

3.5.1 Animal stocks and handling

We performed experiments on 3- to 4-day-old adults from three laboratory colonies of the fruit fly, *Drosophila melanogaster* (Meigen). The first colony descended from a wild-caught population of 200 females. The second and third colonies came from *rover* and *sitter* stocks of the *foraging* gene isolated from natural populations provided by Marla Sokolowski. We reared, entrained, and tested all flies on a 16 h: 8 h light: dark photoperiod. Transitions between light and dark were immediate. The light-on phase started at 7AM PST. We maintained fly stocks at $25\,^{\circ}\mathrm{C}$ and at a relative humidity of either 30% or 60% on Lewis food medium in standard 250 mL bottles (Lewis, 1960).

Unless otherwise noted, we housed groups of 50 flies in vials (AS-515; Thermo Fisher Scientific, Inc., Waltham, Massachusetts, USA), on a 2 mL aliquot of food from a food medium (Ralph Greenspan, personal communication) consisting of 30 mL Karo dark corn syrup, 15 g sucrose, 15 g Torula yeast (Lake States, Wisconsin, USA), 10 g agar, and 1.0 L distilled water. To help with counting and sorting, we immobilized flies by cooling them to $4\,^{\circ}\mathrm{C}$ on a Peltier stage (Marlow Industries, Inc., Dallas, Texas, USA).

3.5.2 Environmental test chambers

We used a system of hardware and software developed to help automate studying the movement of flies between controlled sensory environments described previously.

3.5.3 Dispersal assay protocol

We introduced groups of 50 flies into the first of two connected chambers. Unless otherwise stated, in all experiments we deprived flies of food, but not water, by transferring 50 flies into single vials containing 2 mL of 0.5% agar for 12 hours preceding a given trial. If an experiment included food, we used the same recipe as we had for rearing. This food was introduced as a small dollop on the top surface and the center region of a 2 mL plug of 0.5% agar. Unless noted, in all chambers we provided access to a 2 mL plug of 0.5% agar to prevent dehydration. We introduced flies into chambers at 9AM and waited 1 hour for them to settle down before starting experiments. We opened gates leading into connected chambers at 10AM and monitored the movements of flies until 4PM. In these and all subsequent experiments, we ran trials during this midday, 6-hour time window to avoid confounding interactions with crepuscular morning and evening peaks in activity.

For all experiments, we ran simultaneous trials in 16 pairs of connected chambers. Within a given experiment, we pooled results from trials run over several days. Unless otherwise indicated, all data within this report were reported as mean $\pm$ s.e.m. exit rates per hour and were averaged over 6 hours for statistical analyses (SPSS, SPSS, Inc., Chicago, Illinois, USA).

3.5.4 Detection assay protocol

We designed this experiment to assess whether flies could perceive the amount of inaccessible food used for the studies of dispersal. We introduced groups of 50 flies into a single chamber and recorded their locations using a camera mounted above each chamber. These flies had been deprived of food, but not water, as described for the dispersal experiments. In each chamber, we placed a quartet of containers, each embedded within holes in a false floor. Each cuvette contained water and food (65 μL of food on top of a 2 mL plug of 0.5% agar), only water (2 mL plug of 0.5% agar), or was empty (Fig. 3.3A, B). We covered all containers with mesh so their contents were inaccessible to flies. In each trial we positioned a cuvette containing water and food opposite to one containing only water, and the two other cuvettes were empty (Fig. 3.3A, B). Between trials we used fresh cuvettes, switched the mesh covers, and rotated the location of the cuvettes to control for the build up of olfactory cues or effects that might bias movement, such as asymmetric geometry or lighting. After allowing the flies to settle for 1 hour, we recorded their position every 5 minutes throughout each experiment, using custom software written in Python (Straw and Dickinson, 2009). We determined the number of flies positioned within specified regions using custom software written in Matlab (Mathworks Natick, MA, USA). We normalized loitering frequencies to take into account that during each trial, chambers contained one cuvette with food and water, one with only water, and two that were empty.

3.5.5 Activity experiments

To test whether a change in the intensity of a fly's general locomotor activity might have contributed to their differences in dispersal, we measured the effects of hunger on

their general locomotor activity using commercially available *Drosophila* Activity Monitors (TriKinetics, Inc., Waltham, Massachusetts, USA). Unless otherwise specified, we reared, housed, entrained, and handled flies, as well as ran experiments, over the same midday, 6-hour time window, as we had in the dispersal and detection experiments. During trials all flies had access only to water. When the channel from the monitor for a particular fly stopped registering events, and continued not registering events throughout the rest of the experiment, we assumed that this marked the death of the fly. We adjusted the calculation for mean activity for each 5 minute period throughout the experiment, taking into account the death of the individuals making up the mean.

3.5.6 Supplementary Table

Table 3.1: Ambient environmental conditions from experiments within this study and from a representative sample of studies published from the 1970s until present on the behavior of *Drosophila melanogaster*

Experiment	Figure	Year	Duration (days)	Temperature (°C)*	% Relative Humidity*
Hunger	3.1	2004	10	25.9±0.2	28.9±8.6
Accessibility of food	3.2	2005	9	23.7±0.7	30.3±4.4
Detection of food	3.2	2008	11	21.3±0.3	41.5±9.4
Foraging gene	Discussion	2005	10	25.7±0.3	43.3±2.7
Literature‡	>1970			24.1±1.8	62.8±9.4

*Mean±s.t.d., n.r.Not recorded, ‡From 62 articles.